

**AMENDMENTS TO THE CLAIMS**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of claims**

1. (Previously Presented) A *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by any gene, which gene harbors at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2.

2. (Cancelled)

3. (Cancelled)

4. (Previously Presented) The *cis*-acting nucleotide sequence according to claim 1 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of the nucleotide sequence denoted by SEQ ID NO:1.

5. (Previously Presented) The *cis*-acting nucleotide sequence according to claim 1 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of the nucleotide sequence as denoted by SEQ ID NO:2.

6. (Previously Presented) The *cis*-acting nucleotide sequence according to claim 5

wherein said gene encodes a protein selected from the group consisting of enzymes, hormones, growth factors, cytokines, structural proteins, industrially applicable proteins, agriculturally applicable proteins, a protein which is a therapeutic product, a protein which is an agricultural product, and a protein which is an industrially applicable product.

7. (Previously Presented) A DNA construct comprising:-

- a) a gene which contains at least one intron;
- b) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, operably linked to said gene; and
- c) optionally further comprising additional control, promoting and regulatory elements,

and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2.

8. (Previously Presented) The DNA construct according to claim 7 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor

necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of the nucleotide sequence as denoted by SEQ ID NO:1.

9. (Previously Presented) The DNA construct according to claim 7 wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of the nucleotide sequence as denoted by SEQ ID NO:2.

10. (Previously Presented) A DNA construct according to any one of claims 7, 8 or 9 wherein said control, promoting and regulatory elements are suitable transcription promoters, transcription enhancers and mRNA destabilizing elements.

11. (Previously Presented) The DNA construct according to claim 7, wherein said gene which contains at least one intron, encodes a protein selected from the group consisting of enzymes, hormones, growth factors, cytokines, structural proteins, industrially applicable proteins, agriculturally applicable proteins, a protein which is a therapeutic product, protein which is an agricultural product, and a protein which is an industrially applicable product.

12. (Previously Presented) The DNA construct according to claim 11 wherein said nucleotide sequence is contained within an exon of said gene.

13. (Previously Presented) The DNA construct according to claim 11 wherein said nucleotide sequence is inserted within an intron of said gene.

14. (Currently Amended) The DNA construct according to claim ~~[[13]]~~ 12 wherein said gene is the human TNF- $\alpha$  gene.

15. (Currently Amended) The DNA construct according to claim 14 being the plasmid pTNF- $\alpha$ , in which said *cis*-acting ~~element~~ nucleotide sequence is contained within an exon of the human TNF- $\alpha$  gene.

16. (Previously Presented) The DNA construct according to claim 15 being the plasmid pTNF- $\alpha$ (3'UTR- $\alpha$ EP).

17. (Previously Presented) The DNA construct according to claim 7 wherein said gene is the human TNF- $\beta$  gene.

18. (Currently Amended) The DNA construct according to claim 17 in which said *cis*-acting ~~element~~ nucleotide sequence is contained within an exon of the human TNF- $\beta$  gene.

19. (Previously Presented) The DNA construct according to claim 18 being the plasmid pTNF- $\beta$  (3'UTR- $\alpha$ ).

20. (Previously Presented) The DNA construct according to claim 18 being the plasmid pTNF- $\beta$ (3'UTR- $\alpha$ EP).

21. (Cancelled)

22. (Currently Amended) The DNA construct according to claim [[14]] 13 wherein the DNA construct is pTNF $\alpha$ ( $\Delta$ 3'UTR)i3EP.

23. (Previously Presented) A vector comprising the *cis*-acting nucleotide sequence according to claim 1 or the DNA construct according to claim 7 and a suitable DNA carrier, capable of transfecting a host cell with said *cis*-acting nucleotide sequence.

24. (Previously Presented) The vector according to claim 23 optionally further comprising additional expression, control, promoting and regulatory elements operably linked thereto.

25. (Previously Presented) The vector according to claim 24 wherein said carrier is salmon sperm DNA.

26. (Previously Presented) The vector according to claim 24 wherein said carrier is viral DNA.

27. (Previously Presented) A host cell transfected with the DNA construct according to claim 22.

28. (Previously Presented) A host cell transfected with the vector according to claim 23.

29. (Currently Amended) A host cell according to claim 27 ~~or 28~~ being a eukaryotic or yeast cell.

30. (Previously Presented) The host cell according to claim 29 being a mammalian hemopoietic cell, fibroblast, epithelial cell, or lymphocyte.

31. (Previously Presented) The host cell according to claim 27 wherein said eukaryotic cell is the baby hamster kidney (BHK-21) cell line or the Chinese hamster ovary (CHO) cell line.

32-46. (Cancelled)

47. (Previously Presented) A composition comprising the expression vector according to claim 23.

48. (Currently Amended) A method ~~of producing a protein~~ for producing a transfected cell capable of producing a protein comprising

a) transfecting a host cell with a DNA construct to give a host cell capable of expressing said protein, wherein said DNA construct comprises a) a gene which contains at least

one intron, wherein said gene encodes said protein; b) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, operably linked to said gene; and c) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2; and

b) culturing the ~~eells~~ cell obtained in (a) under culture conditions amenable to express said protein; and

~~e) isolating said protein from the cell culture obtained in (b).~~

49. (Previously Presented) A method of producing a protein comprising:

a) providing host cells transfected with a DNA construct, which are capable of expressing said protwherein said DNA construct comprises a) a gene which contains at least one intron, wherein said gene encodes said protein; b) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, operably linked to said gene; and

c) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a sequence selected from the group consisting of SEQ ID NO:1, and SEQ ID NO:2;

b) culturing the cells provided in (a) under culture conditions amenable to express said protein; and

c) isolating said protein from the cell culture obtained in (b).

50. (Cancelled)

51. (Previously Presented) A composition comprising the host cell according to claim 30.

52. (Currently Amended) A method of producing a protein comprising:

a) transfecting a host cell with an expression vector to produce a host cell capable of expressing said protein, wherein said expression vector is selected from the group consisting of (1) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by any gene, which gene harbors at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a sequence of SEQ ID NO:1 or SEQ ID NO:2; and (2) a DNA construct comprising (A) a gene which contains at least one intron, wherein said gene encodes said protein; (B) a *cis*-acting nucleotide sequence which is

capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, operably linked to said gene; and (C) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a sequence of SEQ ID NO:1 or SEQ ID NO:2, and a suitable DNA carrier;

[[c]] b) culturing the cells cell obtained in [[(b)]] (a) under culture conditions amenable to express said protein; and

[[d]] c) isolating said protein from the cell culture obtained in [[(c)]] (b).

53. (Previously Presented) A method of producing a protein comprising:

a) providing host cells transfected with an expression vector to produce a host cell capable of expressing said protein, wherein said expression vector is selected from the group consisting of (1) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by any gene, which gene harbors at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a



sequence of SEQ ID NO:1 or SEQ ID NO:2; and (2) a DNA construct comprising (A) a gene which contains at least one intron, wherein said gene encodes said protein; (B) a *cis*-acting nucleotide sequence which is capable of rendering the removal of introns from a precursor transcript encoded by said gene, which gene includes at least one such *cis*-acting nucleotide sequence, occurring during the production of mRNA of said gene, dependent upon activation of a *trans*-acting factor, wherein said *trans*-acting factor being the RNA-activated protein kinase (PKR) which is capable of phosphorylating the  $\alpha$ -subunit of eukaryotic initiation factor 2, operably linked to said gene; and (C) optionally further comprising additional control, promoting and regulatory elements, and wherein said *cis*-acting nucleotide sequence is derived from the 3' untranslated region of the human tumor necrosis factor  $\alpha$  gene (TNF- $\alpha$ -3'UTR) and consists of a sequence of SEQ ID NO:1 or SEQ ID NO:2, and a suitable DNA carrier,

b) culturing the cells provided in (a) under culture conditions amenable to express said protein; and

c) isolating said protein from the cell culture obtained in (b).

54. (New) A host cell according to claim 28 being a eukaryotic or yeast cell.

55. (New) The host cell according to claim 54 being a mammalian hemopoietic cell, fibroblast, epithelial cell, or lymphocyte.

56. (New) A composition comprising the host cell according to claim 55.